Nucleotide Sequence of the *Escherichia coli dnaJ* Gene and Purification of the Gene Product*

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The dnaJ and dnaK genes are essential for replication of Escherichia coli DNA, and they constitute an operon, dnaJ being downstream from dnaK. The amount of the dnaJ protein in E. coli is substantially less than that of the dnaK protein, which is produced abundantly. In order to construct a system that overproduces the dnaJ protein, we started our study by determining the DNA sequence of the entire dnaJ gene, and an operon fusion was constructed by inserting the gene downstream of the λP_L promoter of an expression vector plasmid, pPL-λ. Cells containing the recombinant plasmid produced dnaJ protein amounting to 2% of the total cellular protein when cells were induced. The overproduced protein was purified, and Edman degradation of the protein indicated that the NH2terminal methionine was found to be processed. From the DNA sequence of the dnaJ gene, the processed gene product is composed of 375 amino acid residues, and its molecular weight is calculated to be 40,975.

DNA replication of bacteriophage λ requires functions of the phage-encoded proteins O and P, as well as those supplied by the host cell, Escherichia coli K12 (1, 2). The latter functions include those of the dnaJ and dnaK gene products (3–5), both of which are also essential for cell growth and appear to be related to cellular DNA and RNA synthesis (3, 6, 7). The dnaJ and dnaK genes constitute an operon (6) and are located in a region between thr and leu on the E. coli chromosome (5).

The properties of the dnaK gene product have recently attracted the interest of many investigators. The dnaK protein is identified as one of the heat shock proteins (8), and its DNA sequence is conserved among a wide variety of organisms ranging from procaryotes to humans (9). The dnaK protein possesses both ATPase and autophosphorylating activities (10). It is demonstrated from the in vitro studies cited above that the dnaK gene product directly interacts with the P protein.

On the other hand, information on the dnaJ gene product is very limited. Although the dnaK gene product is produced abundantly in E. coli and the dnaJ gene is located downstream of the dnaK gene constituting an operon, the amount of the dnaJ protein is substantially less than that of the dnaK protein. Therefore, we started our study by determining the

DNA sequence of the *dnaJ* gene and constructing a recombinant plasmid that overproduces the *dnaJ* gene product.

EXPERIMENTAL PROCEDURES

Bacterial and Phage Strains and Plasmids—Recombinant phages $\lambda dnaKdnaJ$ and $\lambda dnaKdnaJ\Delta 27$ were described elsewhere (6). Bacteriophages M13mp10 and M13mp11 and their host strain JM101 were kindly provided by Y. Kuchino (National Cancer Center Research Institute, Tokyo). A plasmid pMCR561 was kindly donated by T. Miki (Yamaguchi University, School of Medicine, Japan) (11). An expression plasmid pPL- λ that carries the P_L promoter and N gene on a 1215-base pair (bp¹) segment of the genome inserted between the EcoRl and BamHI site of pBR322 and its host strain N4830 (12) were obtained from Pharmacia/P-L Biochemicals.

Enzymes and Reagents—Various DNA-modifying and restriction enzymes were commercial products. [a-22P]dATP (>400 Ci/mmol, 1 Ci = 37 GBq) was purchased from Amersham Corp. Dideoxy-NTPs and deoxy-NTPs were obtained from P-L Biochemicals and Sigma, respectively. Other reagents were commercial products of analytical grade.

Estimation of the dnaJ Protein—Samples were electrophoresed on a 2% NaDodSO₄, 12.5% acrylamide, 0.3% N,N'-methylenebisacrylamide slab gel, and the amount of the dnaJ protein was estimated by densitometry of protein bands stained with Coomassie Brilliant Blue. The dnaJ protein band was identified by superimposing on the gel the autoradiogram of the co-migrating dnaJ protein extracted from UV-irradiated cells infected with λdnaJ phages incubated in the presence of [14C]leucine. As noted by Georgopoulos et al. (13), the dnaJ protein behaves anomalously as migration is greatly affected by the acrylamide concentration of the gel. Under the condition employed in this study, the dnaJ protein migrated as a 40-kDa protein.

Construction of a Plasmid pDNAJ-A and an Expression Plasmid pPL-dnaJ-23—Thirty micrograms of λdnaKdnaJ Δ27 DNA was digested with Aval. By comparison of the digest with similar digests of the wild-type \(\lambda \) DNA and from knowledge on the structure of $\lambda dnaKdnaJ\Delta 27$, a 3.33-kilobase pair (kb) fragment was identified as containing the intact dnaJ gene. It was purified by agarose gel electrophoresis and ligated with molar excess of synthetic EcoRI linkers as described previously (14). The resulting fragments were inserted at the EcoRI site of plasmid pMCR561. Plasmid pDNAJ-A is one of the constructs selected randomly among ampicillin-resistant transformants. Thirty-five micrograms of pDNAJ-A was linearlized with Sall and treated with 1.75 units of Bal31 exonuclease at 30 °C for 1.0 min. The mixture was then treated with EcoRI and fractionated by agarose gel electrophoresis, and the 2.65-kb band was extracted. The ends of the DNA fragments were filled in by treating with DNA polymerase I large fragment, and the resulting DNA was inserted into the Hpal site of the pPL-A DNA. The mixture of recombinant plasmids thus obtained was used to transform strain N4830 at 30 °C and ampicillin-resistant colonies were selected. Each transformant was cultured at 30 °C in broth. At a bacterial concentration of about 2 × 10⁸/ml, the culture was transferred to a 42 °C bath. After incubation for 3.5 h, cell extracts were prepared and analyzed by NaDodSO4-polyacrylamide gel electrophoresis. Nine out of 36 transformants examined produced various amounts of the dnaJ protein ranging from 0.3 to 2% of total cellular proteins as estimated

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¹The abbreviations used are: bp, base pair; kb, kilobase pair; NaDodSO₄, sodium dodecyl sulfate; Hepes, 4-(2-hydroxyethyl)-1-pi-perazineethanesulfonic acid.

by densitometry of the stained gel. The clone which produced the largest amount of the dnaJ protein was named pPL-dnaJ-23.

Purification of the dnaJ Protein-Cells of strain N4830 harboring pPL-dnaJ-23 were grown at 30 °C in 2 liters of LB broth containing ampicillin (50 μ g/ml). At a cell density of OD_{560 nm} = 0.4, the culture was transferred to 42 °C and incubated for 4 h. The cells were harvested, suspended in a buffer containing 25 mm Hepes (pH 7.6), 1 mm EDTA, and stored at -70 °C. Cells were lysed as described (15); frozen-cell suspensions were thawed at 4 °C, adjusted to 80 mm KCl, 2 mm dithiothreitol, and 0.3 mg/ml egg white lysozyme was added. The cells were again frozen and thawed, and lysates were sonicated (30 s) three to four times. After removing cell debris by low speed centrifugation $(3,000 \times g, 10 \text{ min})$, the lysates were subjected to a high speed centrifugation $(200,000 \times g, 30 \text{ min})$. The sedimented proteins were suspended in a small volume of 50 mm potassium phosphate at pH 7.0, and NaCl was added to a concentration of 1 M. The mixture was left standing at 0 °C for 60 min, and insoluble materials were removed by centrifugation (200,000 × g, 60 min). The supernatant, which contained a large part of the dnaJ protein, was dialyzed against 50 mm potassium phosphate (pH 7.0), 6 mm βmercaptoethanol. The precipitate which appeared during dialysis was collected by centrifugation (200,000 × g, 60 min) and suspended in the same buffer. Aliquots of this suspension were subjected to Na-DodSO4-polyacrylamide gel electrophoresis. The dnaJ protein was eluted electrophoretically from slices as described (16).

Other Methods—DNA sequencing was done by the dideoxychain termination method of Sanger et al. (17). Restriction fragments were cloned into the M13mp10 or M13mp11 vectors for dideoxy sequencing (18). The procedures for preparing samples for NaDodSO₄-polyacrylamide slab gel electrophoresis were as described by Ames (19). Purified proteins were sequenced by Edman degradation using an automated gas-phase sequencer (Applied Biosystems, Model 470A).

RESULTS

Nucleotide Sequence of the dnaJ Gene—Heteroduplex and complementation analyses indicated that the λdnaKdnaJΔ27 phage retains the dnaJ gene but lacks a 4-kb DNA fragment covering a large part of the dnaK gene and some of the bacterial DNA present in λdnaKdnaJ (6). AvaI digestion of the λdnaKdnaJΔ27 produced a 3.3-kb fragment that is not present in the wild-type λ phage. The fragment was identified as containing the dnaJ gene (Fig. 1) and was cloned into pMCR561 to obtain pDNAJ-A (see "Experimental Procedures"). Digestion of the 3.3-kb fragment with PstI produced 2.4-, 0.45-, and 0.44-kb DNA fragments. Both the 0.45- and 0.44-kb fragments are present in the wild-type λ DNA. The 2.4-kb fragment contains three HindIII sites, one of which is the site in the original vector DNA used to clone the dnaK

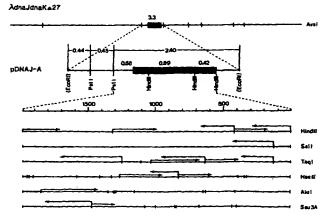


FIG. 1. Strategies employed for DNA sequence analyses of the dnaJ gene of E. coli. Solid bars in \(\lambda \text{naKdnaJ} \text{A27} \) and pDNAJ-A represent the cellular DNA. A 1.9-kb \(PstI\)-HindIII fragment was subcloned into M13mp10 or M13mp11 using restriction enzymes as indicated. \(Arrows\) show direction and extent of each DNA sequencing. About 75% of the final sequence was determined from both strands.

gene (20) and is outside the $\Delta 27$ deletion (6). Therefore, the nucleotide sequence of the remaining region bracketed by the *HindlII* and a *PstI* site was determined (Fig. 1). The sequencing revealed that the bacterial DNA fragment is present in $\lambda dnaKdnaJ\Delta 27$ DNA inserted between positions 20,651 and 27,479 (the *HindlII* site) of the wild-type λ DNA (21).

Fig. 2 shows the complete nucleotide sequence of the 1623-bp long region containing the dnaJ gene. A comparison of our sequence and the sequence of the dnaK gene as published by Bardwell and Craig (9) revealed that the sequence in the region of nucleotides 29–175 is identical to that for the COOH-terminal 49 amino acids of the dnaK protein. The site marked by an arrow in Fig. 2 is the site of the $\Delta 27$ deletion. An open reading frame is found in the region between nucleotides 267 and 1394, providing a coding capacity of 376 amino acids corresponding to a protein having a molecular weight of 41,106. Fig. 2 shows the deduced amino acid sequence.

Purification of the Overproduced dnaJ Protein— The dnaK protein is one of the most abundant protein species produced by E. coli and is easily detected by NaDodSO₄-polyacrylamide gel electrophoretic analysis of total cellular proteins (8). Furthermore, the dnaK protein is known to be a heat shock protein that is produced even more abundantly when the bacteria are incubated at 42 °C (8). Although we have previously presented genetic evidence that the dnaK and dnaJ genes constitute an operon, the dnaK gene being located upstream of the dnaJ gene, the dnaJ protein is difficult to identify on the stained gel even after heat shock treatment.

A close examination of the DNA sequence of the dnaJ gene (Fig. 2) revealed a putative palindromic structure between the Sall site and the initiation codon of the open reading frame. Therefore, in order to construct a plasmid that overproduces the dnaJ protein, the pDNAJ-A DNA was linearized by digestion with Sall, digested by Bal31 exonuclease, and inserted into the expression vector, pPL-\(\lambda\), at the HpaI site (see "Experimental Procedures"). Strain N4830 containing one such recombinant plasmid, named pPL-dnaJ-23, produced dnaJ protein amounting to 2% of total cellular protein upon heat induction by incubation at 42 °C for 4 h (Fig. 3). When pPL-dnaJ-23 DNA was digested with HaeIII, a 320-bp DNA fragment was detected instead of the 402-bp fragment which would be produced from pPL-\(\lambda\) DNA by the same treatment. Thus, the 82-bp region starting from the Sall site and ending at the center of the putative palindromic structure was removed by the Bal31 digestion. The strain N4830 containing pPL-dnaJ-23 was used to purify the dnaJ protein (see "Experimental Procedures").

When the cell extracts were centrifuged and separated into soluble and insoluble fractions, most of the dnaJ protein was recovered in the insoluble fraction. The protein was eluted from the pellet at high concentrations of salts such as NaCl, KCl, or NH₄Cl; the addition of NaCl at a concentration of 1 M solubilized more than 80% of the dnaJ protein present in the precipitate. Dialysis of the eluate against a low concentration of NaCl (0.05 M) resulted in the appearance of a precipitate that is enriched for the dnaJ protein. Further purification of the protein was achieved by NaDodSO₄-polyacrylamide gel electrophoresis and electrophoretic elution of the protein from the gel slices. This final preparation was nearly homogeneous, as shown in Fig. 3, lane 9.

Automated Edman degradation of the purified dnaJ protein revealed Ala-Lys-Gln-Asp-Tyr- as the amino-terminal sequence of the protein. The amino-terminal methionine was not detected, presumably due to in situ processing which is known to occur in many proteins.

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Glu Leu Ala Glu Val Ser Gln Lys Leu Met Glu 1le Ala Gln Gln Gln AAGCTTCCGTAACATCGGCGAAATTCTG GAA CTG GCA CAG GTT TCC CAG AAA CTG ATG GAA ATC GCC CAG CAG CAA
 His Ala Gln Gln Gln Thr Ala Gly Ala Asp Ala Ser Ala Asn Asn Ala Lys Asp Asp Asp Val Val Asp Ala Glu
CAT GCC CAG CAG CAG ACT GCC GGT GCT GAT GCT TCT GCA AAC AAC GCG AAA GAT GAC GAT GTT GTC GAC GCT GAA
 Phe Glu Glu Val Lys Asp Lys Lys end
TIT GAA GAA GTC AMA GAC AMA AMA TAM TCGCCCTATAMACGGGTAMTTATACTGACACGGGCGAAGGGGAATTTCCTCTCCGCCCGTGC
NET ALE LYS GIN ASP TYP TYP GIU ILE LEU GIY VAL SEP LYS THE ALE GIU GIU
ATTCATCIAGGGGCAATTTAAAAAAG
ATG GCT AAG CAA GAT TAT TAC GAG ATT TTA GGC GIT TCC AAA ACA GCG GAA GAG
Arg Glu ILE Arg Lys Ale Typ Lys Arg Leu Ale Met Lys Typ His Pro Asp Arg Asn Gln Gly Asp Lys Glu Ale
CGT GAA ATC AGA AAG GCC TAC AAA CGC CTG GCC ATG AAA TAC CAC CCG GAC CGT AAC CAG GGT GAC AAA GAG GCC
                                                                                                                                                                                                     320
Glu Ala Lys Phe Lys Glu Ile Lys Glu Ala Tyr Glu Val Leu Thr Asp Ser Gln Lys Arg Ala Ala Tyr Asp Gln GAG GCG AAA TTT AAA GAG ATC AAG GAA GCT TAT GAA GTT CTG ACC GAC TGC CAA AAA CGT GCG GCA TAC GAT CAG
                                                                                                                                                                                                     470
 TYT GIY HIS ALA ALA PHE GIU GIN GIY GIY MET GIY GIY GIY GIY PHE GIY GIY GIY ALA ASP PHE SET ASP ILE
TAT GGT CAT GCT GCG TTT GAG CAA GGT GGC ATG GGC GGC GGC GGT TTT GGC GGC GGC GAC GAC TTC AGC GAT ATT
Phe Gly Asp Val Phe Gly Asp Tie Phe Gly Gly Gly Arg Gly Arg Gln Arg Ala Ala Arg Gly Ala Asp Leu Arg
TTT GGT GAC GTT TTC GGC GAT ATT TTT GGC GGC GGA CGT GGT CGT CAA CGT GCG GCG CGC GGT GCT GAT TTA CGC
TYR ASN MET GIU LEU THR LEU GIU GIU ALS VAI ARG GIY VAI THR LYS GIU ILS ARG ILS PRO THR LEU GIU GIU
TAT AAC ATG GAG CTC ACC CTC GAA GAA GCT GTA CGT GGC GTG ACC AAA GAG ATC CGC ATT CCG ACT CTG GAA GAG
Cys Asp Val Cys His Cly Ser Cly Ala Lys Pro Cly Thr Gln Pro Gin Thr Cys Pro Thr Cys His Gly Ser Cly Tot GAC GIT IGC CAC GGT AGC GGT GGA AAA CCA GGT AGC CGG CAG ACT TGT CCG ACC TGT CAT GGT TCT GGT
GIN VAL GIN MET ATG GIN GLY PHE PHE ALA VAL GIN GIN THE CYS PTO HIS CYS GIN GLY ATG GLY THE LEU ILE
CAG GTG CAG ATG CGC CAG GGA TTC TTC GCT GTA CAG CAG ACC TGT CCA CAC TGT CAG GGC CGC GGT ACG CTG ATC
 Lys Asp Pro Cys Asn Lys Cys His Gly Ris Gly Arg Val Glu Arg Ser Lys Thr Leu Ser Val Lys Ile Pro Ala
AAA GAT CCG TGC AAC AAA TGT CAT GGT CAT GGT CGT GTT GAG CGC AGC AAA ACG CTG TCC GTT AAA ATC CCG GCA
GIY VOI AND THE GIY AND AFF ILE AFF LEU ALG GIY GIU GIY GIU ALG GIY GIU HIS GIY ALG PEO ALG GIY ASP
GGG GTG GAC ACT GGA GAC CGC ATC CGT CTT GCG GGC GAA GGT GAA GCG GGC GAG CAT GGC GCA CCG GCA GGC GAT
Leu Tyr Val Gln Val Gln Val Lys Gln His Pro Ile Phe Glu Arg Glu Gly Asn Asn Leu Tyr Cys Glu Val Pro CTG TAC GTT CAG GTT CAG GTT AAA CAG CAC CCG ATT TTC GAG CGT GAA GGC AAC AAC CTG TAT TGC GAA GTC CCG
Ile Asn Phe Ala Met Ala Ala Leu Gly Gly Glu Ile Glu Val Pro Thr Leu Asp Gly Arg Val Lys Leu Lys Val ATC AAC TTC GCT ATG GCG GCG GCG GGT GGC GAA ATC GAA GTA CCG ACC CTT GAT GGT CGC GTC AAA CTG AAA GTG
Pro Gly Glu Thr Gln Thr Gly Lys Leu Phe Arg Met Arg Gly Lys Gly Val Lys Ser Val Arg Gly Gly Ala Gln CCT GGC GAA ACC CAG ACC GGT AAG CTA TTC CGT ATG CGC GGT AAA GGC GTC AAG TCT GTC CGC GGT GGC GAA CCA
GIY ASP Leu Leu Cys Arg Val Val Val Glu Thr Pro Val Gly Leu Asn Glu Arg Gln Lys Gln Leu Leu Gln Glu
GGT GAT TTG CTG TGC CGC GTT GTC GTC GAA ACA CCG GTA GGC CTG AAC GAA AGG CAG AAA CAG CTG CTG CAA GAG
Leu Gin Glu Ser Phe Gly Gly Pro Thr Gly Glu His Asn Ser Pro Arg Ser Lys Ser Phe Phe Asp Gly Val Lys CTG CAA GAA AGC TTC GGT GGT GGC CCA ACC GGC GAC CAC AAC AGC CCG CGC TCA AAG AGC TTC TTT GAT GGT GTG AAG 1370
 Lys Phe Phe Asp Asp Leu Thr Arg end

ANG TIT TIT GAC GAC CTG ACC CGC TAA CCTCCCCAAAAGCCTGCGTGGGCAGGCCTGGGTAAAAATAGGGTGCGTTGAAGATATGCG 1459
 AGCACCTGTAAAGTGGCGGGGATCACTCCCATAAGCGCTAACTTAAGGGTTGTGGTATTTACGCCTGATATGATTTAACGTGCCGATGAATTACTCTCAC 1558
 GATAACTGGTCAGCAATTCTGGCCCATATTGGTAAGCCCGAAGAACTGGATACTTCGGCACGTAA
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Fig. 2. DNA sequence of the dnaJ gene and its flanking regions. The position indicated by the vertical arrow shows the site of the Δ27 deletion introduced into the λdnaKdnaJ phage (6). The inverted repeats in the DNA sequence are indicated by horizontal arrows. The putative promoter and ribosome binding sequences are underlined. The open reading frame is underscribed by corresponding amino acid sequences.

DISCUSSION

In this study, we described the nucleotide sequence of the dnaJ gene of E. coli, purification of the overproduced dnaJ protein, and determination of its NH₂-terminal amino acid sequence.

The initiation codon for dnaJ is located 88 bp downstream of the termination codon of the dnaK gene. Saito and Uchida (6) have reported that transcription of the dnaJ gene is initiated either from the promoter for the dnaK gene or from a weak promoter immediately upstream of the dnaJ gene. The most likely candidate for the second promoter sequence is TATACTG corresponding to the -10 region, located between nucleotides 223 and 229 (Fig. 2). However, assuming that this is the -10 region, sequences corresponding to the -35 region are not obvious. AAGACA, commonly accepted as the consensus sequence for the -35 region (22), appears at positions 189-194. No typical ribosome binding sequence is found at the expected position upstream from the given initiation codon of dnaJ. The sequence AGGGG 14-18 bases upstream from the initiation codon might function as the site of ribosome binding. Another feature we have noticed about the region upstream from the dnaJ gene was the existence of a strong palindromic structure, as indicated in Fig. 2.

The structure might function as an attenuation, resulting

in reduced synthesis of the dnaJ protein. In constructing an operon fusion, pPL-dnaJ-23, to overproduce the dnaJ protein, sequences preceding the dnaJ gene were deleted to varying extents with endonuclease Bal31, and the clone producing the maximum amount of the dnaJ protein was selected. During the course of the selection, we have noticed that different recombinant plasmids constructed in this manner synthesized the protein in different amounts. Detailed analysis of the structure of these recombinant plasmids might provide an insight into the role of the palindromic region mentioned above and the signal responsible for the reduced production of the dnaJ protein as compared to the abundant production of the dnaK protein in E. coli.

Another palindromic region 12 bases downstream from the termination codon of the *dnaJ* gene was noticed. It may be intriguing to speculate that the region might be a termination signal for mRNA synthesis. However, there is no T cluster immediately downstream from the palindrome as is seen in typical ρ -independent termination signals.

Edman degradation of the purified dnaJ protein indicated that the terminal methionine residue is processed in vivo. Therefore, the processed dnaJ protein is composed of 375 amino acid residues, and its molecular weight is calculated to be 40,975. The amino acid composition shows that the protein

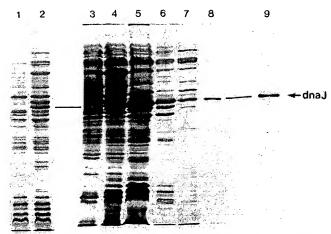


FIG. 3. Purification of the dnaJ protein overproduced in N4830 cells transformed by pPL-dnaJ-23. Cells were induced and extracts were fractionated as described in "Experimental Procedures." Aliquots of fractions at each step were analyzed by NaDodSO₄-polyacrylamide gel electrophoresis and stained with Coomassie Brilliant Blue. Lanes 1 and 2 are the proteins in uninduced and induced cells, respectively. Lanes 3-5 correspond to the proteins in the lysozyme supernatant, fluffy precipitate, and tight precipitate of the centrifuged samples (see text), respectively. Lanes 6 is the 1 M sodium chloride extract of the lane 5 sample. Lanes 7 and 8 are the supernatant and precipitate fractions, respectively, of the sodium chloride extract after dialysis against a buffer containing a low concentration of the salt. Lane 9 is the protein eluted from the acrylamide gel band.



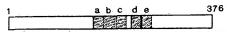


Fig. 4. Homologous amino acid sequences tandemly duplicated within the *dnaJ* protein, as predicted from the DNA sequence of the gene (Fig. 2).

is very rich in glycine (53 glycine residues) and basic amino acids (27 arginine, 27 lysine, and 10 histidine residues). A computer survey for internal homology of the amino acid sequence of the protein revealed that a segment composed of 16 amino acid is duplicated tandemly four or five times (Fig.

4). A search of the protein sequence data base (NBRF/PIR) disclosed that similar tandem duplication of the sequence $Cys \cdot Cys \cdot Gly \cdot Gly$ also occurs within the regulatory protein Q of the λ phage between amino acid residues 128 and 161 of the protein. However, the biological meaning of the occurrence of the sequence is not clear at present.

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